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Recently introduced foods as new allergenic sources: Sensitisation to Goji berries (*Lycium barbarum*)

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ABSTRACT

Goji berries (GB) have been introduced in Western diet. Preliminary reports have demonstrated its allergenic capacity. The objectives of the study were to investigate the frequency of sensitisation and the allergens involved. 566 individuals, with respiratory or cutaneous symptoms were skin-prick tested with GB extract. Thirty three were positive (5.8%). 94% were sensitised to other allergens. Specific IgE to GB, peach, tomato and nut-mix was measured. Thirteen individuals from 24 available sera (54.2%) had positive specific IgE. 92.3% of GB positive patients were positive to peach. Seven individuals recognised 8 bands and six recognised a 7 kDa band. This band was identified as a LTP by MS/MS. Cross-reactivity was demonstrated with tomato, tobacco, nutmix, Artemisia pollen and purified Lyc e 3 and Pru p 3. GB are a new allergenic source with high prevalence of sensitisation. LTP seems to be the major allergen involved in sensitisation and cross-reactivity.

1. Introduction

The international food trade is growing continuously, led by the sophisticated preservation technology and rapid transport methods which make foods safer and more attractive to the consumer. Additionally, consumers' tastes and food habits are changing, becoming more varied, and stimulating the demand for traditional and new foods from other regions, attractive not only for their culinary characteristics but also for their novel properties: so-called "functional foods" (Roberfroid, 2002).

Goji berry (*Lycium barbarum*), also known as wolfberry, belongs to the taxonomical family of Solanaceae and can be considered as one of those foods. It plays an important role in traditional Chinese medicine, mainly related with the capacity to enhance the immune system, help eyesight, protect the liver, and improve circulation (Potterat, 2010).

Although the plant was introduced in Europe as ornamental tree during the XVI century, its fruits were never considered part of the Western diet before the XXI. Goji berries are nutritionally rich, containing various vitamins, minerals, antioxidants, and amino acids (Yao et al., 2011) and have been cited as a very healthy food. These have been some of the reasons behind the rapid spread of its consumption in recent years. Different studies have indeed demonstrated beneficial medical properties in age-related diseases (Chang & So, 2008) and cancer (Gan, Zhang, Yang, & Xu, 2004). Polysaccharides from *L. barbarum* also induce maturation of dendritic cells with strong immunogenicity enhancing Th1 and Th2 response (Chen, Lu, Srinivasan, Tan, & Chan, 2009) and its anticancer and immunomodulatory effects have recently been reviewed (Tang et al., in press). These properties make them a good supplement for people whose immunity may be low or suppressed, such as those

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with cancer, chronic infection, and aging. In contrast, adverse reactions provoked by Goji berry consumption (Arroyo-Martinez, Sáenz, Arias, & Acosta, 2011) or by interaction with the drug warfarin (Lam, Elmer, & Mohutsky, 2001; Leung, Hung, Hui, & Chan, 2008; Rivera, Ferro, Bursua, & Gerber 2012) have been reported.

The first indexed cases of allergic symptoms following Goji berry ingestion, one of them anaphylaxis, were described in two allergic patients (Monzón Ballarín, López-Matas, Sáenz Abad, Pérez-Cinto, & Carnés, 2011). Both individuals had positive specific IgE to Goji berry extract. More recently, another study described allergic symptoms in 5 individuals, finding a high number of asymptomatic sensitisation to Goji berries among a selected group of plant food allergic subjects (mostly sensitised to nonspecific lipid transfer proteins – LTPs) also from Spain (Larramendi et al., 2012). LTPs are ubiquitous allergens present in plants, not only in Rosaceae fruits (Pastorello et al., 1994; Sanchez-Monge, Lombardero, Garcia-Selles, Barber, & Salcedo, 1999) but also in foods from different taxonomical groups (Flinterman et al., 2008; Gómez-Gómez et al., 2010; Hartz et al., 2007; Krause et al., 2009; López-Matas et al., 2011). They are responsible for a large number of allergic sensitisations, mainly in patients residing in the Mediterranean area, ranging from oral allergy symptoms (Asero, 1999) to anaphylactic reactions (Asero et al., 2009; Salcedo, Sanchez-Monge, Diaz-Perales, Garcia-Casado, & Barber, 2004).

The objectives of the study were to characterise Goji Berry extracts, to describe the allergens involved and to investigate cross-reactivity with other foods. As a secondary objectives we investigated the clinical characteristics of individuals, the prevalence of sensitisation in our area of study, and the allergenic profile of patients sensitised to this recently introduced food in Western European Countries.

2. Material and methods

2.1. Patient population

A multicenter observational prospective study was performed between May and October 2010 in the following centers, all of them located in South-East Spain: Complejo Hospitalario Universitario de Cartagena (Murcia), Hospital Marina Baixa (Villajoyosa, Alicante), Hospital de la Vega Baja (Orihuela, Alicante), Hospital Virgen de la Arrixaca (Murcia), and Centro de Especialidades El Españoleto (Játiva, Valencia). Patients attending these centers for the first time, for respiratory and/or cutaneous symptoms, and/or suspicion of plant food allergy, and with clinical indication for the performance of skin prick test (SPT) to common allergens were included. All patients gave written consent to participate in the study. Subjects not fulfilling the criteria or declining consent were excluded. The study was approved by the Ethics Committee of the Complejo Hospitalario Universitario de Cartagena (Murcia).

2.2. Extract manufacturing

Goji berry fruits were purchased at a local market, homogenised in PBS 0.01 M pH 7.4 and extracted for 4 h at 4 °C. The content was centrifuged and the supernatant collected, dialyzed, filtered, frozen, and freeze dried. The protein content was measured by the Lowry-Biuret method (Sigma, St. Louis, Mo., USA).

Non-standardized SPT were prepared with the extract at 10 mg of freeze-dried material per millilitre.

2.3. Skin prick test. Prevalence of sensitisation

Skin prick tests were performed on 566 patients with the same battery of aeroallergens, consisting of standardized extracts of mites (*Dermatophagoides pteronyssinus*, and *Dermatophagoides farinae*), pollens (*Olea europaea*, grass mix, *Artemisia vulgaris*, *Cupressus arizonica*, *Plantago lanceolata*, *Salsola kali*, *Chenopodium album*, *Parietaria judaica*, *Platanus hybrida*, *Betula alba*, and *Phoenix dactytifera*), molds (*Alternaria alternate* and *Cladosporium herbarum*), animal dander (cat and dog), tomato peel, peach peel, and purified LTP (Pru p 3, and Cor a 8) (Laboratorios LETI S.L, Madrid, Spain), date palm pollen semi-purified profilin (containing 50 μg/ml of Pho d 2), and a polcalcin-enriched extract from date palm pollen (ALK, Madrid, Spain). Patients reporting symptoms to specific foods were SPT with those foods.

All individuals were also skin prick tested with the Goji berry extract. Wheal sizes were measured by papulometry. This technique consists of the measurement of the wheal area using the PC Draft software (Microspot, Maidstone, UK) and results are expressed in mm^2.

Serum samples were collected from positive patients who gave their consent. Subjects were asked about their knowledge and previous consumption of Goji berries. An open challenge test was offered to all Goji berry symptomatic subjects.

2.4. Protein profile (SDS–PAGE and 2D electrophoresis)

SDS–PAGE analysis was used to determine the protein profile of the extract. Sixty micrograms of Goji berry proteins were loaded in SDS–PAGE gels with 2.67% C, 15% T acrylamide under reducing conditions and stained with Biosafe Coomassie (Bio-Rad Laboratories, Hercules, CA, USA).

Protein profile was also analysed by 2D electrophoresis. The extract was purified and concentrated with ammonium sulfate in two different steps until reaching the saturation percentage (40% and 80%), then maintained at 4 °C overnight. Thereafter, the sample was centrifuged, the pellet collected and then reconstituted in ultra-purified water. Concentrated extract was washed using ReadyPrep 2-D Cleanup Kit (BioRad), following the manufacturer's instructions. Proteins were separated according to their isoelectric point in ReadyStrip IPG Strips (BioRad) in a pH range between 3 and 10, using Protean IEF Cell (BioRad). After the first dimension, the strip was equilibrated with the ReadyPrep 2-D Kit buffers (BioRad) and proteins were separated in the second dimension according to their molecular weight. Gel was stained with Oriole solution (BioRad) following the manufacturer's instructions.

2.5. Specific IgE (CAP)

A total of 700 μg of protein from Goji berry extract were previously labelled using a biotin kit (Roche Diagnostics, Mannheim, Germany) and were used in the solid phase. Aliquots of 50 μl of Goji berry biotin-labelled extract were incubated in streptavidin uniCAPs (ThermoFisher Scientific) for 30 min. Specific IgE to commercial mixture of nuts (fx1: peanut, hazelnut, Brazil nut, almond, and coconut), tomato (f25), and peach (f95) was also determined. The experiment was performed by the ImmunoCAP 100E system (ThermoFisher Scientific).

2.6. Allergenic profile

The allergenic profiles of the Goji berry extract were studied by immunoblot, using ImmunoCAP positive individual sera. Briefly, after SDS–PAGE, the proteins on the gel were electrotransferred to an Immobilon®-P membrane (Millipore, Bedford, Mass., USA) and dried at room temperature. Thereafter, membranes were incubated overnight with the sera diluted 2/3 in PBS. After incubation with monoclonal antihuman-IgE-PO (Ingenasa, Madrid, Spain), the reaction was developed with luminol (BioRad) and visualised by chemiluminescence. A serum pool was prepared with identical